Evaluation of Antioxidant Activity and Bioefficacy of Encapsulated Silver Nanoparticles against *Aedes aegypti*

Synopsis

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Dengue is a mosquito-borne viral disease transmitted mainly by *Aedes aegypti* and *Aedes albopictus*. Recently, dengue transmission has strongly increased in urban and semi-urban tropical areas worldwide, becoming a major international public health concern. Over 2.5 billion people are now at the risk from dengue. The World Health Organization estimates that there may be 50–100 millions dengue cases worldwide every year. There are four distinct, but closely related, serotypes of the virus that cause dengue (DEN-1, DEN-2, DEN-3, and DEN-4). Recovery from infection by one provides lifelong immunity against that particular serotype. However, cross-immunity to the other serotypes after recovery is only partial and temporary (WHO, 2012). Currently, there is no specific treatment for dengue and its prevention and control solely depends on effective vector control measures.

Conventional chemical insecticides had been an effective means of controlling mosquito vectors especially during the outbreaks of dengue however, their heavy usage leads to the development of resistance. In the last decades enormous efforts have been made to explore the efficacy of botanicals against dengue vectors and have been evaluated for their potential as adulticidal, larvicidal, oviposition deterrent, growth inhibitors, adult repellents etc. although the studies are restricted to laboratory conditions only. There are few reports on the transovarial transmission of dengue virus (Ahmad *et al*., 2007; Angel and Joshi, 2008; Costa *et al.*, 2017) and dengue virus has also been discovered from the field collected *A. aegypti* larval stages hence, for effective management of dengue transmission nanoscale larvicides are warranted to kill the larvae as well as virus.

Nanobiotechnology is receiving immense attention in recent years and an active area of research in contemporary material science. The potential of nanoparticles and nanomaterials

is emerging rapidly. Silver in nanocrystalline form has found excellent applications in the field of high sensitivity bio molecular detection, disease and chemical diagnostics, antimicrobial and therapeutic compounds. Metallic silver is known to fight infections since ancient times and has been exploited for biomedical applications owing to its antimicrobial properties.

Nanoparticles have unique physicochemical properties which not only render them suitable for tremendous applications in various fields but also provides strong reactivity which, if not stablilized may generate oxidative stress. For this, surface stabilization is required. In the present study, green route of surface stabilized silver nanoparticle synthesis will be followed which may be confirmed through the evaluation of their antioxidant potential. Nanotoxicity is a major concern in rapidly growing era of nanomaterial development for diverse applications. Hence, in this study one objective is dedicated for toxicity assessment which will be done for screening and developing safer and biocompatible encapsulated silver nanoparticles. For this purpose, toxic effects of synthesized nanoparticles if any, would be evaluated through cell viability assay on selected mammalian cell lines.

In the past all life cycle stages have been targeted for effective and integrated vector control. However, in the present study major focus would be on the control of larval stages as there are reports on the transovarial transmission of dengue virus which may be responsible for the outbreaks of dengue. Extensive research has been conducted on the mosquito larvicidal potential of plant mediated silver nanoparticles however, no reports are available on the synthesis and larvicidal efficacy of encapsulated silver nanoparticles which is the major objective of this study for effective management of dengue vectors. In this study, we propose to develop encapsulated nanoparticles as a novel tool against *Aedes aegypti*, the primary vector of dengue.

REVIEW OF LITERATURE

Aedes aegypti is a cosmo tropical mosquito which serves as an important vector for infectious diseases like dengue, Chikungunya and newly introduced Zika virus. Dengue is endemic in more than 100 countries and its incidence has increased 30-fold in the past 50 years. Due to the increased development of resistance in mosquito vectors against synthetic insecticides and chemical drugs, plant based insecticides serve as promising alternatives for their effective control. In the literature review, antioxidant properties of plants to be used for silver nanoparticle preparation as well as efficacy of silver nanoparticles specifically against *A. aegypti* have been included.

Study of antioxidant properties of potential antiviral candidates for controlling viral replication seems necessary as this would help in combating the oxidative stress generated due to viral infections thus enhancing effectiveness of the nanoparticles. Past decade has evinced researches on the evaluation of antioxidant activity of phyto extracts for diverse applications. This review will specifically focus on the plants being used in this research for justifying their selection. Montelongo *et al.* (2010) tested antioxidant activity in banana peel extracts through evaluating extraction conditions and related bioactive compounds. In the same year, Angayarkanni *et al.* (2010) tested antioxidant potential and radical scavenging potential of *Amorphophallus paeoniifolius* for their phenolic content. In many papers it has been reported that fruit peels have more nutrients than the pulp and ripe fruit (Sundaram *et al.*, 2011). They evaluated antioxidant activity and protective effect of Banana peel against oxidative hemolysis of human erythrocyte at different stages of ripening. Similarly, Fatemeh *et al.* (2012) investigated total phenolics, flavonoids and antioxidant activity of Banana pulp and peel flours. Umar *et al.* (2012) studied anti-inflammatory and antioxidant activity of *Trachyspermum ammi* seeds in collagen induced arthritis in rats. Singh *et al.* (2013) conducted phytochemical analysis of peel of *Amorphophallus paeoniifolius.* Chatterjee *et al.*

(2013) evaluated antioxidant activity of essential oil from ajwain seeds. Mashkor (2014) studied phenolic content and antioxidant activity of fenugreek seed extract. Santos *et al.* (2014) performed chemical characterization of the peel and seed from Papaya cultivars. Suttirak *et al.* (2014) conducted *in vitro* antioxidant properties of mangosteen peel extract. Lawrence *et al.,* (2015) explored antioxidant activity of lemon grass essential oil grown in north Indian plains. Mandal *et al.* (2015) investigated the antioxidant property of *Carica papaya* leaf extracts from Mizoram, Northeast India. Said *et al.* (2016) studied antibacterial and antioxidant properties of hydrodistillated extract of *Eucalyptus globulus* fruits. Salla *et al.* (2016) studied antioxidant and apoptotic activity of Papaya peel extracts in hepG2 cells. Pavithra *et al.* (2017) evaluated nutritional properties of Papaya peel. Singh and Ahmad (2017) extracted essential oil of *Trachyspermum ammi* seeds using supercritical CO_2 and studied its antioxidant properties.

In recent years nanotechnology has attained considerable attention due to its vast biomedical applications and promising technology for developing novel nanomedicines. Specifically silver nanoparticles have been synthesized by diverse routes and explored for several unique properties. There are reports which show that silver nanoparticles have antiviral, antifungal (Ales *et al*., 2009), anticancerous activity (Chung *et al.,* 2016). Their antiviral activities have been explored against several viruses including Dengue (Lara *et al.,* 2010; Etemadzade *et al.,* 2016; Yang *et al.,* 2016)

A number of strategies have been used to control mosquitoes including chemical and biological methods. However, effective management has been achieved through the use of conventional approaches which not only proved to be toxic but also led to the development of resistance in mosquitoes. Literature survey shows that several plants and plant parts have been screened for biosynthesis of silver nanoparticles and their evaluation for mosquito vector control.

Philip *et al.* (2011) biosynthesized gold and silver nanoparticles using leaves of Krishna tulsi (*Ociumum sanctum).* Kamlakannan *et al.* (2011) evaluated toxicity of *Acalypha indicia* and *Achyranthes aspera* bark extract against *Aedes aegypti*. Use of synthetic insecticides to control vector mosquitoes has caused physiological resistance and adverse environmental effects in addition to high operational cost. Insecticides of synthesized natural products for vector control have been a priority in this area. Gnanadesigan *et al.* (2011) identified the larvicidal activities of silver nano particles synthesised with *Rhizophora mucronata* leaf extract against the larvae of *Aedes aegypti* and *Culex quinquefasciatus*. A biological method was used to synthesize stable silver nanoparticles that were tested as mosquito larvicides against *Aedes aegypti*, *Anopheles stephensi* and *Culex quinquefasciatus*. Arjunan *et al.* (2012) studied Green synthesis of silver nanoparticles for the control of mosquito vectors of malaria, filariasis and dengue.

Boarse *et al.* (2013) documented photosynthesized silver nanoparticles as mosquito biolarvicide agent against *Aedes aegypti* and *Anopheles stephensi*. According to Dhayananthaprabhu *et al*. (2013) microorganisms such as bacteria, fungi and actinomycetes have ability for synthesizing metal nanoparticles. Nemati *et al*. (2013) have studied the cytotoxic properties of some medicinal plant extracts. Kuppsamy *et al*. (2014) have studied nanoparticals as therapeutic target for Malaria, Cancer, HIV, Hepatitis and many others. Mondal *et al.* (2014) presented a report on green synthesis of silver nanoparticles while, Suresh *et al.* (2014) synthesized silver nanoparticles biologically using *Delphinium denudatum* root extract and evaluated antibacterial and mosquito larvicidal activities. In the same year, Priya *et al.* (2014) also synthesized silver nanoparticles by green approach using *calotropis gigantea* and their potential mosquito larvicidal property. The adulticidal activity of silver nanoparticles determined by Veerakumar *et al.* (2014) using *Feronia elephantum* plant leaf extract against adults of *Anopheles stephensi*, *Aedes aegypti* and *Culex quinquefasciatus*. Guerrero *et al*. (2015) have studied the toxicity of Mexican native plant

extracts against larvae of *Aedes aegypti*. As a potential alternative to synthetic insecticides, the use of silver nanoparticles synthesized using *Phyllanthus niruri* leaf extract for mosquito control was performed by Suresh *et al.* (2015). Sujitha *et al.* (2015) proposed the green-synthesis of silver nanoparticles as a novel and effective tool against the dengue serotype DEN-2 and its primary vector *Aedes aegypti*. Chung *et al.* (2016) have synthesized AgNPs from plants and studied their characteristic properties and therapeutic applications. Velayutham *et al.* (2016) evaluated larvicidal activity of synthesized silver nanoparticles using isoamyl acetate identified in *Annona squamosa* leaves against *Aedes aegypti* and *Culex quinquefasciatus*. Most recently, Malabadi *et al.* (2017) documented current updates on the use of *Carica papaya* leaf extract as a potential herbal medicine against dengue virus disease. Frederico *et al.* (2017) reviewed on anti-viral effects of medicinal plants in the management of dengue. Kumar *et al*. (2017) used Photo-induced and phytomediated synthesis of silver nanoparticles using *Derris trifoliata* leaf extract and its larvicidal activity against *A. aegypti*.

In the past all life cycle stages have been targeted for effective and integrated vector control. However, the present study will focus on the control of larval stages as there are reports on the transovarial transmission of dengue virus (Ahmad *et al*., 2007; Angel and Joshi, 2008; Costa *et al*., 2017). Extensive research is been conducted on the mosquito larvicidal potential of plant mediated silver nanoparticles however, no report is available on the synthesis and larvicidal efficacy of encapsulated silver nanoparticles which is the major objective of this study for effective management of dengue vectors.

OBJECTIVES

The major objective of the study is the synthesis of encapsulated silver nanoparticles with enhanced mosquito larvicidal activity and least toxicity against non target organisms. For ensuring the synthesis of stabilized nanoparticles antioxidant activity would be studies while for safer development of encapsulated silver nanoparticles toxicity assessment would be studied in mammalian cell lines as a non target model. Hence, present study involves following objectives:

- Synthesis and characterization of encapsulated silver nanoparticles
- Evaluation of antioxidant activity of encapsulated silver nanoparticles
- Bioefficacy of encapsulated nanoparticles against dengue vector *Aedes aegypti*
- Toxicity assessment of encapsulated nanoparticles in HEK 293 mammalian cell lines

MATERIALS AND METHODS

MATERIALS

A) Experimental animal: *Aedes aegypti* (Linn.1762)

A. aegypti is a Dipteran insect of family Culicidae which is prevalent in the tropics and sub-tropic areas. *A. aegypti* is found in at least 23 countries with concentrations in Northern Brazil and Southeast Asia including India. *A. aegypti* is extremely common in areas lacking piped water systems with dependence on stored water. *A. aegypti* eggs are deposited in man made plastic containers which are their preferred breeding sites.

A. aegypti is a small, dark mosquito measuring 4 to 7 mm in length apart from white 'spots' on the body and head regions and white rings on the legs. The thorax is decorated with a white 'Lyre' shape of which the 'chords' are two dull yellow lines. Females are larger than males, and can be distinguished by small palps tipped with silver or white scales. Larvae can be identified by the presence of pitch forked shaped comb scales arranged in a single row.

Aedes aegypti is a holometabolous insect as it passes through a complete metamorphosis with different stages representing a complex life-cycle for 8 -10 days. Females lay eggs in the side walls of water containers, which are white initially and later become black. *Aedes* larvae mainly feed on microorganisms, algae and organic matter and grow from first to fourth instars by shedding their skins thrice, subsequently converting into pupal stage through metamorphosis. Pupa is a non feeding stage and finally emeges into adult stage. The life span of adult mosquito ranges from two weeks to a month depending on the weather conditions. Male mosquitoes feed on flower nectar and they do not bite human beings while female mosquitoes are haematophagus and feed on human blood. *A. aegypti* is considered as an medically important insect as it serves as a vector for the transmission of several diseases such as Dengue, Yellow fever, Chikungunya, West Nile fever and recently introduced Zika virus.

B) Plant material

For the synthesis of silver nanoparticles, thiol rich and saponin rich plant parts have been chosen after conducting a pilot study against dengue vector. For encapsulation of AgNPs, essential oils will be used. Both are described here briefly:

- **Thiol rich botanicals:** Peels of a variety of fruits and plants are gaining attention as a natural source of polyphenols and bioactive compounds, which possess various beneficial effects on human health. In most of the cases the antioxidative compounds were found to be generally higher in the peel than in the pulp and in the green than in the ripe components. It is known that plant by-products are important sources of sugars, minerals, organic acids, fiber and phenolic compounds that have a wide range of pharmacological activities, which include antitumor, antiviral, antibacterial, cardioprotective, and antimutagenic activities

Musa acuminate L. commonly called as Banana, is a member of family Musaceae which is non woody gigantic herb. Antioxidant activities have been detected in peel portion of Banana fruit from unripe to ripe stages which could be a very inexpensive source of extracts rich in phenolic compounds than that of banana pulps.

Carica papaya L. commonly known as Papaya, belongs to the family of Caricaceae. It is an herbaceous plant that grows in tropical and subtropical countries. A large amount of waste such as peels and seeds is generated as waste by product from this fruit which is an economically attractive raw material for AgNPs synthesis which is otherwise a problem for waste management. Phenolic compounds are important constituents exhibiting antioxidant activity by inactivating lipid free radicals.

- **Saponin rich botanicals**: *Trigonella foenum graecum* commonly called as Fenugreek is an annual leguminous plant of *Fabaceae* family. Its seeds and green leaves are used as food and known to possess medicinal properties. However, the most useful part is their seeds which are golden-yellow in colour, small in size, hard and have four-faced stone like structure. The biological and pharmacological actions of fenugreek seeds are mostly attributed to the

variety of its bioactive chemical constituents that serve as raw materials for the manufacture of various hormonal and therapeutic drugs.

Trachyspermum ammi is an annual herb of family Apiaceae. It is commonly called as Ajwain or carum and its leaves and fruits (often called as seeds) are most commonly used parts. Ajwain is used as medicinal plant in traditional Ayurvedic medicine primarily for stomach disorders, antispasmodic, stimulant and tonic. Its biological properties have also been explored as an antioxidant, antibacterial, antifungal, anthelmintic etc.

- **Essential oils**: Essential oils are the volatile compounds with strong aroma and considered as by products of plant metabolism. These plant secretions are mainly derived from leaves, flowers, stem etc. which could be extracted through steam or hydro distillation. Monoterpenes, sesquiterpenes, phenols etc. are the major constituents of EOs which render them various biological functions such as attractant and repellent properties and responsible for their antibacterial, antifungal, insecticidal, antioxidant activity. In the present study, EOs obtained through steam distillation such as Lemon grass oil, *Eucalyptus* oil and Ajwain oil would be used for evaluating their impact on mosquitoes and Dengue virus as these EOs are known to possess activity against mosquitoes and few viruses. GC-MS analysis of EOs will be done for phytochemical screening. In this study they will be used to enhance the activity of nanoparticles through encapsulation.

METHODOLOGY:

1) Preparation of aqueous plant extract

For the synthesis of aqueous plant extract from experimental fruit peels and seeds, 5-25% extract would be prepared. Fruits will be procured from the local market, washed with purified water and freshly peeled off to get the peels, finely chopped, mixed with 100 ml MilliQ water and gently heated and cooled. Likewise seeds will be gently washed, crushed and mixed with MilliQ water and heated to get 5-10% extract as their extract is very viscous.

The extract would be filtered through Whatman filter No. 1 and 0.25 µm millipore filter which can be used and stored for about one week in refrigerator.

2) Green synthesis of AgNPs

Before sysnthesizing encapsulated AgNPs, green synthesis of AgNPs is required. For this, 1 mM aqueous silver nitrate ($AgNO_3$) will be prepared in a 250ml of dark bottle. Dark brown bottle will be used for the preparation of this solution to prevent the degradation. Herbal extract will be added in silver nitrate solution with different combinations and ratios (1:9, 2:8 and 3:7) for stabilization of nanoparticles using thiol rich and saponin rich plant materials at room temperature. The changes in colour of colloidal solutions would indicate the formation of AgNPs.

3) Synthesis of encapsulated silver nanoparticles

For encapsulation of AgNPs, a novel approach will be followed for enhancing their activity. First of all, nanoemulsions would be synthesized with medicinally important essential oils like, *Eucalyptus* oil, Lemongrass and Ajwain oils by using high energy method i.e. ultrasonication. Polyoxyethylene sorbitan monooleate (Tween 80) and Milli Q water will be used alongwith essential oils (Oil and surfactant will be mixed in different ratio to optimize the protocol for nanodroplet size) for the preparation of nanoemulsions. Tween 80 will be used as the surfactant or emulsifier as it is the non toxic, hydrophilic, stable against pH variation and ionic strength and compatibility with other ingredients. The resultant emulsion will be sonicated for 1h with the help of ultrasonicator. Finally, AgNPs will be solubilized in aqueous phase with milli Q water and encapsulated using best optimized and stable nanoemulsion ratios. Entrapment efficiency (%) of the nanoparticles in essential oil nanoemulsions will also be calculated. A pilot study for the synthesis of encapsulated AgNPs has already been done in the laboratory.

4) Characterization of synthesized AgNPs

The initial characterization of the synthesized AgNPs would be carried out using UV–Vis spectroscopy. UV – VIS Spectrophotometric analysis will be done for monitoring the reduction of pure silver ions by measuring the reaction medium at different time interval by diluting a small aliquot of the sample with milli Q water. For further characterization harvesting of AgNPs is needed which would be done by centrifugation at 8000rpm for 20 minutes followed by redispersion of pellet in milli Q water. Finally, the pellet would be oven dried and collected for characterization of silver nanoparticles, Scanning electron microscopy (SEM) would be done to check surface morphology, transmission electron microscopy (TEM) would be done for studying size, morphology etc. While, X-ray diffraction (XRD) analysis will be required to study crystalline nature of AgNPs. The purified AgNPs will be examined for the presence of functional groups and biomolecules using Fourier Transform Infrared (FTIR). Encapsulated nanoparticles will be characterized for droplet size and zeta potential through NanoZetasizer and their stability will be checked at various temperatures. Characterization would be done at Chemistry Department, DEI, Advance Instrumentation Research Facility, JNU and AIIMS, New Delhi.

5) **Evaluation of antioxidant activity of synthesized nanoparticles**

Nanoparticles have unique physicochemical properties which not only render them suitable for tremendous applications in various fields but also provides strong reactivity which, if not stablilized may generate oxidative stress. For this surface stabilization is required. In the present study, green route of nanoparticles synthesis will be followed which may be confirmed through the evaluation of their antioxidant potential.

- **DPPH antioxidant activity assay:** To check antioxidant activity of nanoparticles, free radical scavenging activity assay, (DPPH) would be conducted according to Zhao *et al*. (2006). The antioxidant properties of thiol and saponin functionalized AgNPs would be measured by monitoring their quenching capacity against synthetically stable DPPH radical.

Briefly, solution containing 1.5 ml of different concentrations (10-100ppm) of AgNPs and encapsulated AgNPs will be added to 1.5 ml of 0.25 mM DPPH. After that solution mixtures will be incubated for 30 min at room temperature and change in colour would be observed with the help of uv-vis spectrophotometer at 517 nm.

- **Hydroxyl radical scavenging assay**: OH scavenging activity of AgNPs would be measured according to Yu *et al.* (2004) Reaction mixture containing 60µl of 1.0mM FeCl2, 90µl of 1mM 1, 10-phenanthroline, 2.4ml of 0.2M phosphate buffer (pH 7.8), 150µl of 0.17M H_2O_2, and 1.5ml of different concentrations (10-100 ppm) of AgNPs and encapsulated AgNPs would be incubated at room temperature for 5 minutes. After incubation, scavenging activity of the AgNPs will be measured spectrophotometrically.
- **Nitric Oxide antioxidant activity assay**: NO free radical scavenging assay will be done by using Kang *et al*. (2006) method. Briefly, NO scavenging assay will be performed by mixing 1.0 ml of 5.0 mM sodium nitroprusside with 1.0 ml of different concentration of thiol (10-100ppm) AgNPs and encapsulated AgNPs. Then mixtures would be incubated for 60 min at room temperature. After incubation, an equal amount of Griess reagent (1% sulphanilamide, 2% phosphoric acid, and 0.1% napthylethylene diamine dihydrochloride) will be added to the reaction mixture and absorbance would be measured at 546 nm by uv-vis spectrophotometer.
- **Hydrogen peroxide scavenging activity assay**: H_2O_2 scavenging activity of nanoparticles would be evaluated through replacement titration according to the method of Zhao et al. (2006). 1.0 ml of H_2O_2 (0.1 mM) will be mixed with 1.0 ml of different concentrations (10-100ppm) of AgNPs and encapsulated AgNPs. To this 2 drops of 3% ammonium molybdate, 10 ml of 2 M H_2SO_4 and 7.0 ml of 1.8 M KI would be added. The reaction mixture will be titrated with 5.09 mM $Na_2S_2O_3$ until the disappearance of yellow colour.

Percent Free radical scavenging activity in the above assays would be calculated using the following formula: [(C-T/C)] x 100, where C is absorbance of the control and T for the test samples. In all the assays Ascorbic acid will be used as standard antioxidant agent.

6) Efficacy of nanoparticles against *A. aegypti* larvae

- **Collection and Rearing of Larvae:** *A. aegypti* larvae will be collected from natural oviposition sites through standard dipping procedures and brought to the laboratory and separated into various instar larvae. Larvae would be maintained in dechlorinated tap water and provided with standard fish food. Third instar larvae would be used for the experiments.

- **Mosquito Larvicidal Bioassay:** Larvicidal bioassay of AgNPs would be carried out according to standard procedure of World Health Organization (WHO, 2005). Effectiveness of synthesized AgNPs would be tested on 20 mosquito larvae with 200ml of distilled water. Bioassays will be performed for third instar larvae at least six concentrations of AgNPs, essential oil nanoemulsions and encapsulated AgNPs for better comparison. Stock solution will be prepared from which appropriate volume will be added to the beakers to obtain desired target concentrations. Besides this, a negative control with distilled water and a positive control with permethrin would be run in parallel with experimental setup. Each test will include five replicates for each concentration. Mortality will be recorded after 24 hours exposure and expressed as LC_{50} and LC_{90} which would be calculated by Probit analysis (Finney, 1971).

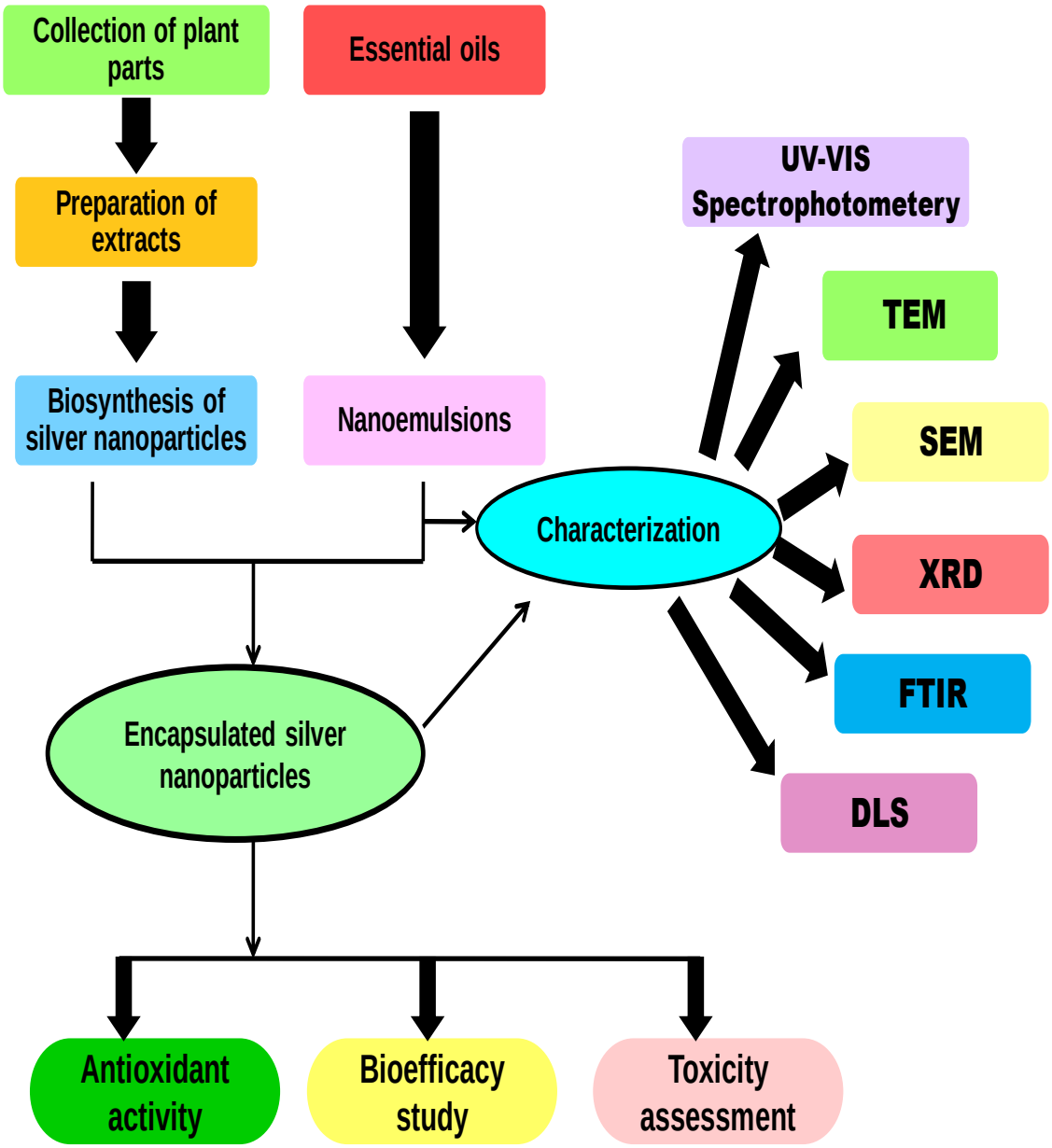
7) Toxicity assessment of nanoparticles in HEK 293 mammalian cell lines

Nanotoxicity is a major concern in rapidly growing era of nanomaterial development for diverse applications. Hence, in this study one objective is dedicated for toxicity assessment which will be done for screening and developing safer and biocompatible mosquitocidal encapsulated nanoparticles. For this purpose, toxic effects of synthesized nanopreparations if any, would be evaluated through cell viability assay (MTT assay) following the basic protocol of Mosmann (1983) on selected mammalian cell lines

namely, Human embryonic kidney cells 293 (HEK 293) which will be considered as a model non target screening of nanoparticles. Cell viability will be checked by the use of MTT (3-(4, 5- dimethylthiazolyl-2)-2, 5- diphenyltetrazolium bromide) for assessment of cytotoxicity. MTT is a yellow coloured dye, which is reduced into violet coloured formazan crystals by the activity of mitochondrial succinate dehydrogenase enzyme in viable cells. For this, HEK 293 cells would be cultured in 96-well microtiter plates using DMEM along with 10% FBS and 1% Penicillin streptomycin as a culture medium and incubated for overnight at 37^{o} C. The morphology of cells will be monitored with inverted microscope before exposure to synthesized nanoparticles. Cells would be treated with varying concentrations of different nano particles for 24 and 48 hours. At the end of the treatment period, 10µl of MTT will be added to each well and the plates will be incubated for 3 h in a dark chamber and supernatant from each well would be aspirated. Finally, 100µl of DMSO will be added to dissolve the formazan crystals. Optical density would be recorded at 570 nm with microplate reader and percent cell viability will be calculated for IC_{50} values as follows: % Cell Viability= (Absorbance of treated cells/Absorbance of untreated cell) x100. This part of the study would be done at ICGEB, New Delhi.

8) **Statistical analysis:** Data will be expressed in terms of Mean ± SD values. Larvicidal susceptibility will be expressed in terms of LC_{50}, LC_{90}, and calculated by probit analysis (Finney, 1971) after correcting control mortality by using Abbott's formula (Abbott's, 1925) **T-C/100-CX100,** Where, T = % Mortality of test organism, C = % Mortality of control. One way ANOVA will be used to analyze mean differences between experimental groups using statistical software.

EXPERIMENTAL DESIGN



PLAN OF WORK

Phase I

1. Synthesis of encapsulated silver nanoparticles:
    - Preparation of aqueous plant extract
    - Green synthesis of silver nanoparticles
    - Synthesis of encapsulated silver nanoparticles
2. Characterization of nanoformulations through :
    - TEM, SEM, XRD, FTIR, DLS etc.

Phase II

1. Evaluation of antioxidant activity of synthesized nanoparticles:
    - DPPH antioxidant activity assay
    - Hydroxyl radical scavenging assay
    - Nitric Oxide antioxidant activity assay
    - Hydrogen peroxide scavenging assay

Phase III

1. Toxicity assessment of nanoparticles in HEK 293 mammalian cell lines :
    - MTT assay (Mosmann, 1983)

Phase IV

1. Efficacy of nanoparticles against *Aedes aegypti* larvae.

PRELIMINARY WORK DONE

- Literature survey
- Pilot study for selection of experimental plants through screening of various plant parts.
- Optimisation of protocols

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